Effect of mouth rinses on the elution of monomers from dental composite Materials

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ABSTRACT

Mouth rinses have been known to exert some effects on the surface of the dental composite materials. This in vitro study evaluated the effect of mouth rinses (with and without alcohol) on the substances released from composite materials. Eighty samples (diameter: 4.5 mm and thickness: 2 mm) of two composite materials (Ceram X™ and Filtek™ Supreme XT) were prepared. Four different mouth rinses were evaluated: two contained alcohol (Listerine: 21.6% and One drop only: 76%) and two were alcohol-free (Chlorhexamed® 0.2% and Meridol®). Ten (10) specimens of each composite were stored in each mixture of mouth rinse/human saliva for 1 h. These solutions were analyzed by LC-MS/MS for detection of BisGMA, TEGDMA, Bisphenol A and two types of UDMA. There were no release of substances by any of the tested composite samples independent of the mouth rinse used and only traces of UDMA 2 under the quantification limit (0.1 µg/ml) was detected. The use of mouth rinses independent of the amount of alcohol it contains seems not to affect the elution of substances from composite materials.

Key words: Dental materials, composite resin, HPLC, mass spectrometry, dimethacrylate.

INTRODUCTION

The rapid development of dental restorative materials and techniques in the past decades in combination with the modern concept of the minimal invasive therapy in restorative dentistry have resulted to increased use of composite resins in the daily clinical practice. The advantages of the composite materials are based on their rapid polymerization, ability to bond with dental surfaces, excellent mechanical properties and aesthetic appearance.

Basically, dental composite resins consist of a resin matrix, inorganic filler and a coupling agent. Common monomers used in the resin matrix are BisGMA (bisphenol A glycol dimethacrylate), UDMA (urethane dimethacrylate), TEGDMA (triethylene glycol dimethacrylate) and BisEMA (bisphenol A ethoxylated dimethacrylate). The degree of polymerization affects the physical properties and the clinical performance of resin composite materials (Ferracane, 1985; Chung, 1990; Yoon et al., 2002).

However, besides the physical and mechanical properties of the composite materials, their biocompatibility plays an important role regarding the success of the dental restoration. The release of substances, such as BisGMA, TEGDMA, HEMA (2-hydroxethyl methacrylate) and UDMA from dental composite materials were studied extensively in this literature (Spahl et al., 1998; Naçaci et al., 2006; Polydorou et al., 2007, 2009; Tabatabaei et al., 2009; Kopperud et al., 2010). Resin monomers like TEGDMA, BisGMA and HEMA have been shown to induce cytotoxicity and apoptosis in human dental pulp cells (Goldberg, 2008;
Table 1: Composite materials used.

<table>
<thead>
<tr>
<th>Material</th>
<th>Type</th>
<th>Main monomer composition</th>
<th>Manufacturer</th>
</tr>
</thead>
<tbody>
<tr>
<td>Filtek™ Supreme XT</td>
<td>Universal Restorative – Nanohybrid</td>
<td>BisGMA, TEGDMA, UDMA and BisEMA</td>
<td>3M ESPE Dental Products, Seefeld, Germany.</td>
</tr>
<tr>
<td>(shade A2B)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Ceram X™ (shade M2)</td>
<td>&quot;Ormocer&quot; Universal Nano-Ceramic Restorative</td>
<td>Methacrylate modified polysiloxane and dimethacrylate resin</td>
<td>Dentsply DeTrey GmbH, Konstanz, Germany.</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
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</tr>
</tbody>
</table>

Spagnuolo et al., 2004; Krifka et al., 2011; Chang et al., 2010.

The release of Bisphenol A from polymerized composite materials was proven in several studies (Polydorou et al., 2009; Tabatabaee et al., 2009; Kopperud et al., 2010) raising a major concern about its possible implications on human health, as it can mimic the effects of steroid natural hormones and is known to act as estrogen-receptor antagonist causing endocrine disruption. The release of substances is thought to be combined with an incomplete polymerization (Chung, 1990; Yoon et al., 2002).

Moreover, the exposure of composite restorations to plaque acids, food and salivary enzymes in the oral environment which causes material softening (Asmussen, 1984; Ferracane and Marker, 1992) and chemical degradation can also lead to the release of various substances from dental composite restorations such as residual monomers, oligomers and other degradation products (Geurtsen, 1988; Finer and Santerre, 2004; Polydorou et al., 2007; Kopperud et al., 2010; Borges et al., 2011).

Substances released from composite materials can diffuse through the dentine to reach the pulp tissue (Goldberg, 2008) or affect the periodontal tissues after being eluted in the oral cavity, increasing the concern about possible detrimental effects on oral soft tissues.

The use of mouth rinses in the oral cavity increased during the past years due to the wide spectrum of their indications. Mouth rinses contain various substances such as water, salts, preservatives, anti-microbial agents and sometimes alcohol. The difference in their composition may affect the pH (Moran et al., 2000) and also its effect on the physical-mechanical properties of dental composite restorations that might exist in the oral cavity (Gurdal et al., 2002; Yap et al., 2003; Cavalcanti et al., 2005; Gurgan et al., 2008; Almeida et al., 2010; de Silva et al., 2014).

Mouth rinses containing alcohol have been shown to reduce the micro hardness of composite materials (Cavalcanti et al., 2005), increase their surface roughness (de Silva et al., 2014) and the solubility of composite materials (Almeida et al., 2010; Ozer et al., 2014).

However, no information exists in this literature pertaining to the possible effect of mouth rinses on the elution of substances from dental composite materials. The aim of the present in vitro study was to evaluate the effect of mouth rinses on the elution of substances from two different dental composite materials and the hypothesis tested was that mouth rinses containing alcohol result in higher amount of monomers compared to those without alcohol.

**MATERIALS AND METHODS**

In the present in vitro study, two different composite materials were used: a nanohybrid composite resin [Filtek™ Supreme XT (3M ESPE Dental Products Seefeld, Germany)] and an ormocer [Ceram X™ (Dentsply DeTrey GmbH Konstanz, Germany)]. Table 1 shows detailed information about the composition of the composite materials.

Chlorhexamed® 0.2% (without alcohol), Listerine® Cool Citrus, Meridol® Mouth rinse (without alcohol) and One Drop Only® were the four different mouth rinses evaluated and Table 2 shows detailed information about the ingredients of the mouth rinses and manufacturers. Each tested mouth rinse was diluted in human saliva and the solutions prepared were used for the storage of the composite specimens: 20 ml of Chlorhexamed® Fluid, Listerine® Cool Citrus and Meridol® mouth rinse were each diluted separately in 2 ml of saliva. For the solution of one drop only,® 5 drops were mixed with 19.75 ml of water and 2 ml of human saliva, as this product is a concentrated one.

From each composite material, four groups (n=10) of specimens were produced for each tested mouth rinse. The samples were prepared using forms allowing the production of standardized cylindrical specimens (diameter: 4.5 mm and thickness: 2 mm) and the forms were positioned on a transparent plastic matrix strip lying on a glass plate. They were then filled with the respective composite materials and the samples built up in one increment. After inserting the materials into the discs, a transparent plastic matrix strip (Kerr Hawe, Switzerland) was placed on top in order to avoid an oxygen-inhibited superficial layer.

Additionally, a glass slide was used in order to flatten the surface. The samples of the composite materials were
Table 2: Mouth rinses tested.

<table>
<thead>
<tr>
<th>Name</th>
<th>Amount of alcohol (%)</th>
<th>Ingredients</th>
<th>Manufacturer</th>
</tr>
</thead>
<tbody>
<tr>
<td>Chlorhexamed® Fluid 0.2%</td>
<td>0</td>
<td>Chlorhexidine (D-glucurate), glycerol, macroglycerolhydroxystearate, Aroma, cochineal red (E124) and water: Eucalyptol, Menthol, Methyl salicylate, Thymol, water, alcohol, sorbitol solution, flavouring, poloxamer 407, benzoic acid, zinc chloride, sucralose, sodium benzoate, FD and C yellow no. 6, FD and C red no. 40.</td>
<td>GlaxoSmithKline Consumer Healthcare GmbH and Co. KG, Berlin, Germany.</td>
</tr>
<tr>
<td>Meridol® Mouthrinse</td>
<td>0</td>
<td>Amine fluoride, zinc fluoride, water, xylitol, PVP, PEG-40, hydrogenated castor oil, aroma, stannous fluoride, sodium saccharine and CL 42051.</td>
<td>GAB GmbH, Lorrach, Germany.</td>
</tr>
<tr>
<td>One Drop Only® (concentrate)</td>
<td>73</td>
<td>Alcohol, water, Menthol, Mentha piperita, Thymol, Thymus vulgaris, aroma, eugenol, limonene and benzyl benzoate.</td>
<td>One Drop Only GmbH, Berlin, Germany.</td>
</tr>
</tbody>
</table>

Table 3: Monomers used as reference standards.

<table>
<thead>
<tr>
<th>Compound</th>
<th>Name</th>
<th>Chemical type</th>
<th>Molecular weight*</th>
<th>CAS-number</th>
</tr>
</thead>
<tbody>
<tr>
<td>BisGMA</td>
<td>Bisphenol A glycol dimethacrylate</td>
<td>C₂₀H₃₆O₈</td>
<td>513.0</td>
<td>1565-94-2</td>
</tr>
<tr>
<td>TEGDMA</td>
<td>Triethylene glycol dimethacrylate</td>
<td>C₁₃H₂₂O₆</td>
<td>286.32</td>
<td>109-16-0</td>
</tr>
<tr>
<td>UDMA 1</td>
<td>Urethane dimethacrylate Product</td>
<td>C₁₃H₁₄O₆N₂</td>
<td>498.0</td>
<td>-</td>
</tr>
<tr>
<td>UDMA 2</td>
<td>Urethane dimethacrylate</td>
<td>C₁₃H₁₆N₂O₈</td>
<td>470.56</td>
<td>41137-60-4 or 72869-86-4</td>
</tr>
<tr>
<td>BPA</td>
<td>Bisphenol A</td>
<td>C₁₃H₁₄O₂</td>
<td>228.29</td>
<td>80-05-7</td>
</tr>
</tbody>
</table>

*Information given by the manufacturers.

delivered using a halogen unit (Elipar® Highlight, 3M ESPE Seefeld, Germany) with a light intensity of 780 to 800 mW/cm². The spectral irradiance was determined using a visible curing light meter (Cure Rite Dentsply, USA) and the samples polymerised for 20 s according to the manufacturers’ instructions. Directly after curing, each sample was immediately immersed in the respective storage medium according to the group they belong. The samples were stored in a dark box at room temperature for one hour after which the storage medium and solution samples (1 ml each) were removed and stored at 4°C in the dark until analysis.

The analyses of samples were performed with a High Performance Liquid Chromatography tandem Mass Spectrometry (LC-MS/MS). A Triple quadrupole mass spectrometer (Model 1200 L) from Varian Inc. combined with an HPLC was used. The separation of the monomers took place with a CC 70/3 Nucleodur 100-3 C18ec HLC-Column (Macherey-Nagel, Duren, Germany) using a gradient program with 0.1% (v/v) formic acid and acetonitrile as solvents.

As reference standards, bisphenol A, BisGMA and two different forms of UDMA, TEGDMA and HEMA were used (Table 3 shows the information on the substances used as reference standards). For the analysis, external calibrations with standards were obtained with the help of the peak areas. Identification of monomers was performed by retention time and MRM experiments in MS/MS mode. The limits of quantification were: 0.1 µg/ml for UDMA, TEGDMA and BisGMA and 0.5 µg/ml for Bisphenol A values beyond this level could not be qualified.

RESULTS AND DISCUSSION

None of the monomers used as reference standards were found to be from Filtek™ Supreme XT and Ceram X™ in any of the four tested mouth rinses. Some traces of UDMA 2 were detected to be eluted from Ceram X™ specimens after storage in one drop only® and from the specimens of Filtek™ Supreme XT after storage in Chlorhexamed® fluid solution. However, these values were beyond the quantification limit of 0.1 µg/ml. Therefore, no statistical analysis could be performed.

In the present in vitro study, two different composite materials were used in order to evaluate the effect of mouth rinses on the elution of monomers, representing two different modern categories of composite materials. Ceram X™ is anOrmocerTM and contains a low amount of monomers while Filtek™ Supreme XT is a nanohybrid
composite material. It was shown that the composition of the composite materials affects the success of polymerization (Christensen et al., 1999; Yap et al., 2003). Ormocers showed a lower release as compared to composite materials with conventional chemistry (Polydorou et al., 2009; Kopperud et al., 2010) indicating a better biocompatibility.

According to the study of Hickel et al. (1998) the advantages of Ormocer include low shrinkage, high abrasion resistance and biocompatibility. Brackett et al. (2007) found that materials with traditional compositions such as Filtek™ Supreme XT can be severely cytotoxic throughout an 8-week interval; however, the biocompatibility of materials with newer chemistries such as Ceram X™ has improved over time of aging in artificial saliva.

The difference in the chemical structure of the composite materials and the variation in the ratio of the filler and monomers have a significant effect on the element release and cytotoxicity level of the materials (Al-Hiyasat et al., 2004). In previous studies, (Polydorou et al., 2009, 2011, 2012; Kopperud et al., 2010) the elution of the same composite materials used in the present study was evaluated under different experimental conditions and periods and it was shown that the ormocer technology results in a lower elution of substances compared to Filtek™ Supreme XT.

However, in the present study with respect to the elution of substances no differences were observed among the two materials as none resulted in any elution of substances, although the same analytical methods were used in the past. Mouth rinses did not cause any release of monomers from the composite materials after exposure for one hour. This short period of storage is probably the reason for the present findings. Storage for one hour in a mixture of saliva and mouth rinse represents a daily use of the mouth rinses for 30 s per day for 120 days (4 months) in the oral cavity which is actually a long period of use for some mouth rinses.

In addition, the amount of the solution used for storage of each composite sample in the present study was higher than the amount of medium used in the previous studies in order to simulate the amount of mouth rinses that come in contact with composite materials in the mouth after daily use and this definitely also has an effect on the positive findings.

In the literature (Gurdal et al., 2002; Cavalcanti et al., 2005; de Silva et al., 2014) 20 ml of mouth rinses were used in order to evaluate its effect on the surface and mechanical properties of composite materials while in another study (Ozer et al., 2014) 10 ml of mouth rinses were used. In the present study, 20 ml of mouth rinses in combination with 2 ml of human saliva was used, representing the mean amount of stimulated saliva produced in healthy objects at the time tested (Nikiforuk, 1985). The use of saliva might have positive effect relating to the elution of monomers.

In the past (Polydorou et al., 2011, 2012), the storage of composite materials in human saliva resulted in low or no elution of substances compared to more aggressive media like ethanol (75%). The short storage time used in the present study in combination with the composition and amount of the saliva used as storage medium are probably the reasons for the present results.

The effect of mouth rinses containing alcohol on the elution of monomers from the composite materials were compared to those that are alcohol-free but no detrimental effects were observed on the composite materials pertaining to the elution of substances by any of the mouth rinses tested. Ethanol has a softening effect on composite materials after attacking their cross-linked network which can result in an easier release of substances. Benetti et al. (2009) after studying the softening and elution of monomers in ethanol detected significant negative correlations between softening and elution in ethanol respectively, and the degree of conversion.

According to Schneider et al. (2008), one of the main effects of ethanol (75%) is weakening of the mechanical properties of the composite materials. Besides these, ethanol (75%) has a solubility parameter which matches that of BisGMA which is often the reason for a higher release of this substance (McKinney and Wu, 1985). The short storage time and the presence of saliva is probably the reason that no difference was found in mouth rinses with and without alcohol.

Furthermore, it was suggested in the past (Lin et al., 2005; Seiss et al, 2009) that esterases containing human saliva are related to composite resin biodegradation and it might be able to affect the stability of monomers such as TEGDMA and HEMA. In the case of low release of substances, a possible degradation of the eluted monomers might have taken place, thereby influencing the findings.

According to Hagio et al. (2006) that tested the degradation of the methacrylates monomers in saliva, it was found that the urethane groups obtained an improved resistance to salivary hydrolysis. This property of the urethane groups might be responsible for the fact that some traces of UDMA could be detected in human saliva. Further research is necessary in order to prove such speculations and evaluate the interaction between the mouth rinses and the substances that might be eluted from the composite materials.

In the literature, in order to evaluate the effect of mouth rinses on the mechanical and physical properties of the composite materials, the storage time of the composite samples in the mouth rinses varies between 12 h (Gurdal et al., 2002; Yap et al., 2003), 2 days (Ozer et al., 2014) or 14 days (Cavalcanti et al., 2005) resulting in significant findings among the studies.

However, the aim of the present study was to evaluate
the effect of mouth rinses on the elution of monomers simulating the clinical conditions as far as possible. Mouth rinses containing alcohol was shown to affect the solubility of composite materials (Almeida et al., 2010; Ozer et al., 2014) after 24 h or 2 days of storage. A longer storage time of the composite samples in the mouth rinses/saliva solutions in the present study might have also resulted in different findings; however, some of the products used in the present study are recommended for a short-term use.

According to the present findings, the hypothesis made at the beginning of the study cannot be accepted. None of the mouth rinses tested showed an effect on the elution of substances indicating that their use does not influence the biocompatibility of the composite restorations, irrespective of the amount of alcohol they contain.

**Conclusion**

Within the limit of the present study, the use of mouth rinses does not have any influence on the elution of substances from dental composite materials for the short-term period evaluated. The use of mouth rinses with or without alcohol seems not to be detrimental in relation to the biocompatibility of the dental restorations.

**REFERENCES**


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